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Treatment efficiency of fish processing wastewater in different types of biological reactors

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ABSTRACT

Fish processing technologies generate a significant amount of organic waste, both solid and liquid. Fish wastewater (FWW), if untreated or ineffectively treated, poses a threat to the aquatic environment.

We investigated efficiency of different types of bioreactors with fixed and moving beds in the treatment of wastewater from fish processing. Pollution levels in raw effluents reached 260–660 mg $O_2 \cdot dm^{-3}$ for BOD_5, 450–950 mg $O_2 \cdot dm^{-3}$ for suspended chemical oxygen demand (SCOD), 16.5–23 mg dm^{-3} for PO_4^{3-}, and 10–18 mg dm^{-3} for NH_4^+. It was typical wastewater such as leachate and wastewater after fish processing. The models of bioreactors (on a laboratory scale) identified ring fixed bed reactors (RFBR) as the most efficient under efficient aeration. Average reduction of COD and BOD_5 in RFBR was 44–80%, and 72–77% respectively, and for nutrients, it ranged from 16 to 34% for PO_4^{3-} and reached 42% for NH_4^+. The other experimental bioreactors were significantly less effective. The application of FRBR in wastewater treatment from fish processing would allow effective reduction of organics pollutants and nutrients concentration in treated effluents.

1. Introduction

Global fish consumption has doubled since 1973, which is the result of population growth and changes in consumer trends. The consumption level will remain at 16.2 and 21.5 kg (capita year)$^{-1}$ for developing and developed countries respectively, up to 2020 (Chowdhury et al., 2010). Fish processing generates industrial wastewater of variable specificity, related to the processing technology and the type of fish (saltwater or freshwater). This type of wastewater contains significant amounts of easily degradable organics (colloids including proteins, lipids and other organic compounds found in fish bodies). Therefore, the effluents are a mixture of organic soluble substances and suspended matter, and their concentrations vary depending on the production processes (Palenzuela-Rollon et al., 2002; Rollon, 2005; Chowdhury et al., 2010). COD and suspended matter concentrations are very variable and show processing facility-depending differences. Selection of wastewater treatment technology requires a preliminary analysis of the production technology as well as the parameters and the amount of generated effluents. The main challenge is a significant level of nutrient load (mainly from protein breakdown) (Islam et al., 2004).

The wastewater pH is usually close to neutral and ranges from 5.5 to 7.6 (on average 6.5) (Technical Report Series FREMP, 1994). The content of suspended matter is 10–30% (on average 25% organic suspension).

The organic loads, measured as biological oxygen demand in 5 days (BOD_5) and carbon-oxygen demand (COD), depend mainly on the type of organic compounds and originate from fish processing. BOD_5 in wastewater from the production of tinned fish usually ranges from 10000 to 50000 mg dm^{-3} (Okumura and Uetana, 1992; Mendez et al., 1995; Najafpour et al., 2006). In the biological step of wastewater treatment, a correct N:P ratio should be 5:1, which determines proper biomass growth of the activated sludge or biofilm growing on bed surface area (Aloui et al., 2009).

The moving biological bed reactors (MBBR) show higher wastewater treatment efficiency than sequencing batch reactors (SBR), and their work is more stable in moderately cold climate zone. They are especially recommended for the treatment of wastewater from various factories and technological processes. MBBR technologies enable a significant reduction in the volume of bioreactors compared to SBR installation operating on the same wastewater parameters (Andreottola

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et al., 2000; Hosseiny and Borghei, 2002; Ødegaard et al., 2004; Falletti et al., 2014).

According to literature data, MBBRs allow for removal of up to 90% of BOD_5 and COD. They are successful in purification of FWW with COD of 5000–10000 (Andreottola et al., 2000; Park et al., 2001; Hosseiny and Borghei, 2002; Aloui et al., 2009). Effective denitrification in the bed (after a startup period) in a wide range of temperatures makes this type of wastewater treatment facility particularly useful in aquacultures and fish hatcheries (Rusten et al., 2006; Chowdhury et al., 2010). Fixed beds, specially designed packages with specific geometric shapes made of synthetic materials (PE, PVC) are mainly used in biofilters for the treatment of small amounts of wastewater (Chmielowski and Ślizowski, 2008; Młynski et al., 2017). Semi-submerged fixed beds (SSFB) belong to the beds most often used on a technical scale. They are often designed as hybrid systems with a sponge, nonwoven materials, and ceramics carriers for biofilm biomass (Sridang et al., 2008; Mazur et al., 2016; Wąsik and Chmielowski, 2017). Such reactors are mainly used in the treatment of wastewater with a low concentration of COD and BOD_5 (in aquacultures pools). SSFB can also be applied to hybrid systems with activated sludge technology.

The main advantages of this type of fixed biological bed involve a relatively low failure rate because of a simple construction, satisfactory efficiency of wastewater treatment, no need for additional aeration, stable operation in temperate climate conditions, and a small amount of excessive sludge. Due to the diversity of wastewaters from fish processing, no universal technological scheme can be used in their treatment. Selection of installations for the treatment of this type of wastewater requires individual analysis. Based on its results we can design an appropriate treatment system (Chowdhury et al., 2010).

The purpose of this study was to examine and better understand FWW treatment process in bioreactors with a different type of biological bed construction.

We compared the treatment effectiveness of the effluents using selected type of bioreactors: MBBR, SBR, fixed beds (FBR) and ring fixed beds (RFBR). Our study involved laboratory scale models of bioreactors and the results are useful in selecting appropriate methods for FWW treatment.

2. Materials and methods

Wastewater used in this experiment was treated until there was a significant improvement in its clarity and satisfactory reduction of COD and BOD_5 was achieved. Oxygen concentration in bioreactors during nitrification phase was checked daily.

2.1. Wastewater preparation

1. Biological material (fish remains from the processing of common carp *Cyprinus carpio* filets) was ground and homogenized without thermal pre-treatment (Fig. 1). Raw wastewater contained various fractions of microscopic particles that were easily separated during the sample filtration for SCOD and BOD_5 measurement.
2. In the second step, an additional thermal pretreatment was introduced, i.e. the pre-homogenized biological material was slowly frozen at $-40\,^{\circ}C$ (Fig. 1).

Both types of substrates were dissolved in water to obtain a wastewater mixture with SCOD load between 800 and 1200 mg $O_2 \cdot dm^{-3}$. Slow freezing of the homogenate (at $-40\,^{\circ}C$) allowed for complete destruction of cell membranes in tissue fragments and release of cytosol fractions.

Fragments of biological beds (2 mm^2) were carefully cut out so as not to destroy the structure of the biofilm. The authors used microscope HUVITZ HRM-300 3D equipped with a camera Lusis HC-20CU and software Panasis 3D Profiler. Observations and measurements of biofilm samples were carried out in bright field mode. The samples were scanned in magnification 20x and the 3D biofilm model were calculated in Panasis 3D software. There were made measurements of morphological parameters of biofilm in randomly selected cros-section of 3D model.

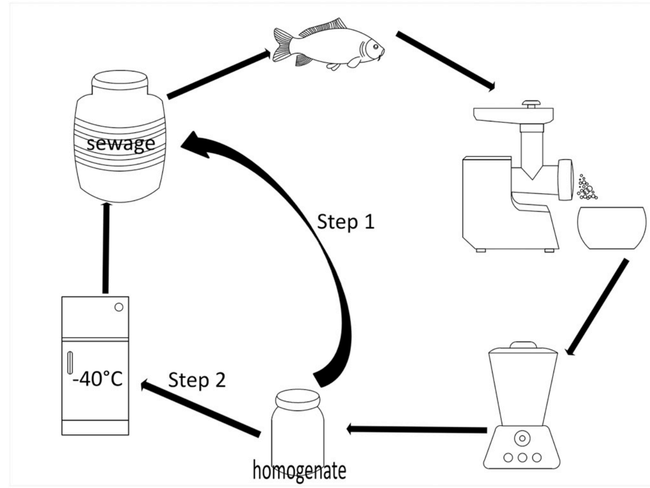

Fig. 1. Scheme of FWW preparation for the treatment process on selected types of biological beds.

The research station involved four groups of experimental bioreactors with different biological bed structure. Each experimental group contained three replicates with the same type of bioreactors located in plastic containers of 30 dm^3 volume.

The bioreactor volume was 1.5 dm^3, and bed capacity 1 dm^3. The systems were equipped with a fine bubble diffuser (Fig. 2). The reactors were filled with the following beds: MBBR, SBR, FBR, RFBR (Fig. 2a, b, c, d).

In MBBR bioreactors the biomass carriers filled 50% of the bed in the reactor chamber. The bioreactors operated in an air-lift column mode and full circulation of carriers was obtained (Fig. 2a). The SBR beds operated in three reactors with activated sludge without biomass carriers (Fig. 2b).

In all the models with fixed bed both stationary (Fig. 3c) and ring constructions (Fig. 3d) in the form of PE corrugated tubes (7 mm in diameter) were used. The tubes were cut and arranged into homogeneous packages and ring structures with spaces between the ring layers. Differences between biological bed construction are presented in Fig. 3.

2.2. Experimental conditions

Two membrane blowers (Hillbow AT 80) aerated the bioreactors, one per two groups of bioreactors. Intense fine-bubble aeration enabled the flow of the effluents through the reactor. The design and location of the suction nozzle ensured full mixing of wastewater into the system (Fig. 3).

The aeration mode was set at 1.5 h of aeration (nitrification stage) and 1.5 h of an anoxic phase (denitrification stage). Oxygen concentration during aeration of the treated FWW was always above 2 mg dm^{-3}. The temperature of treated effluents was 20–22 °C.

Sampling took place after 24, 48, 72, 96 and 120 h of the treatment, from the top of each bioreactor (during aeration stage), in the first stage of the experiment. The last sampling was done after 72 h in the second research campaign.

2.3. Analytical procedures

Collected samples were filtered on the ashless Whatman filters (0.4 μm pore size) for SCOD analysis, or analyzed without filtering for

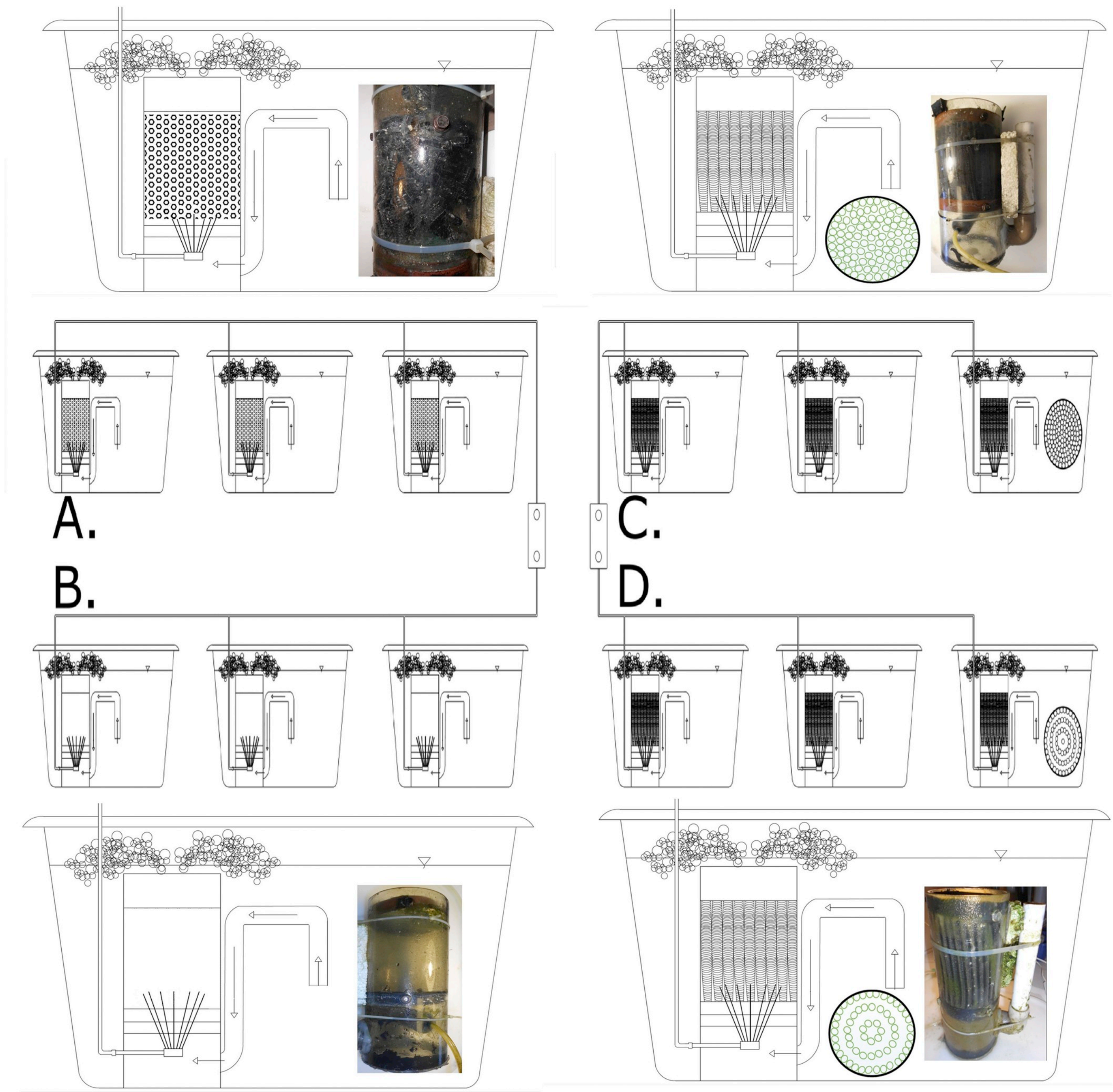

Fig. 2. Laboratory station for testing the treatment efficiency of wastewater from fish processing on biological beds: A. MBBR B. SBR C. FBR D. RFBR.

total chemical oxygen demand (COD_{tot}).

NOVA 60 Spectroquant® photometer was used for analysis of COD, NH_4^+, and PO_4^{3-}.

1. COD was measured according to the procedure of Merck Millipore in NOVA 60 Spectroquant®, 114555 - COD cuvette test, a method for oxygen range of 500–10000 mg $O_2 \cdot dm^{-3}$. The samples were mineralized in a termoreactor at 148 °C for 120 min (Spectroquant NOVA 60. COD - 114541. 2013a).
2. BOD was measured according to the Oxi-top protocol (WTW Company) over five days of incubation of the substrate samples (in dark glass bottles with continuous stirring at 20 °C). The results were multiplied by a factor (x 20), (the range of the expected BOD_5 was 0–800, the volume of wastewater sample = 97 ml). (OxiTop BOD).
3. NH_4^+ ions were analyzed with the spectrophotometer Spectroquant NOVA 60 from Merck. Method PB-06, issue 2 from 2013 (Spectroquant NOVA 60. Ammonium test - 100683. 2013b).
4. PO_4^{3-} ions were measured with the spectrophotometer Spectroquant NOVA 60 from Merck. Method PB-07 issue 2 from 2013 (Spectroquant NOVA 60. Determination of orthophosphate no. 100616. 2013c).

A multifunction device Elmetron CPC-461 was used for the online monitoring of temperature, pH and O_2 concentration (with automatic temperature compensation).

2.4. Statistical analysis

The results were analyzed using Statistica 13.1 software. The differences between experimental groups were calculated based on

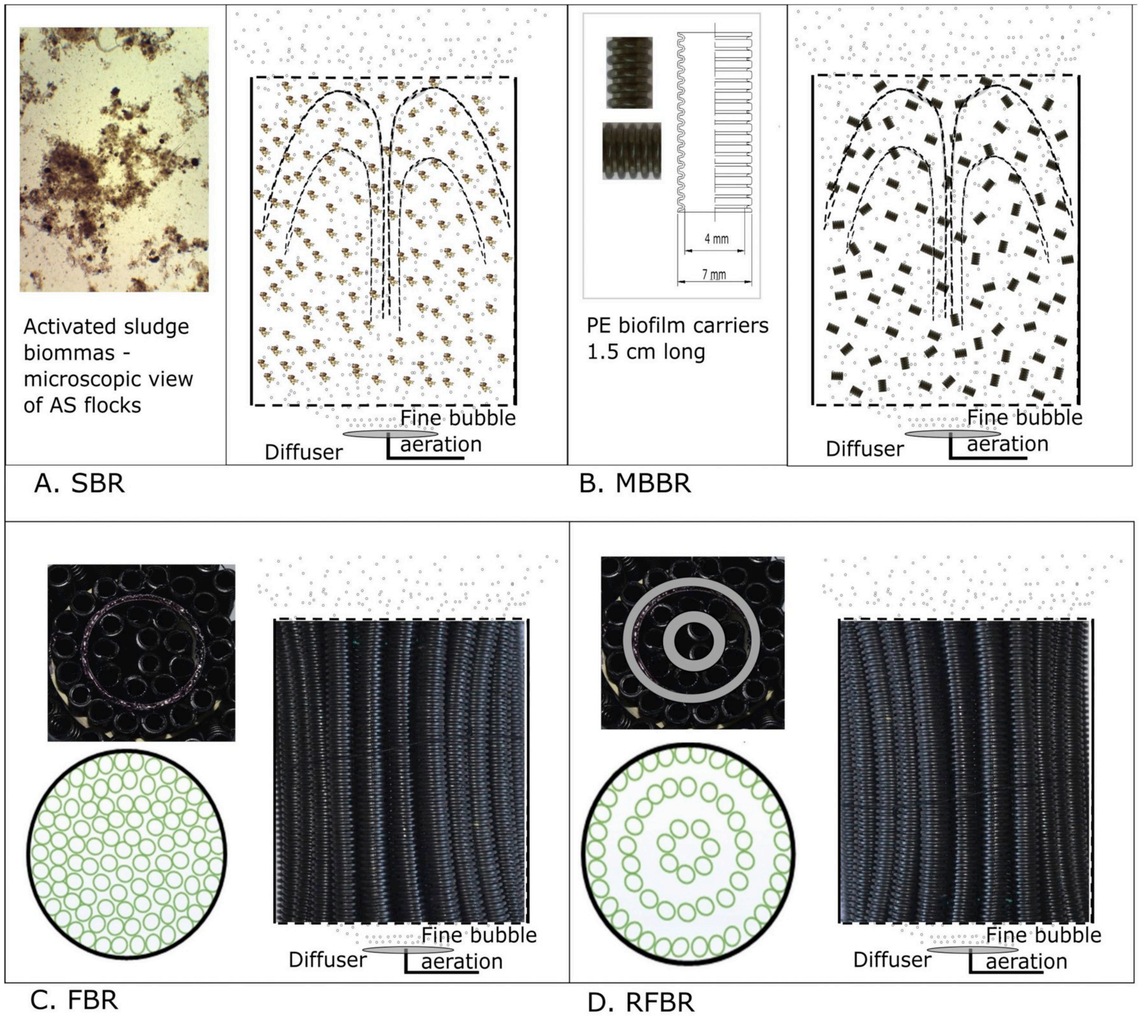

Fig. 3. Diagram of the structure of biological beds used in laboratory models of the bioreactors, along with biofilm carriers: A. MBBR B. SBR C. FBR D. RFBR.

univariate ANOVA and Tukey's post-hoc tests (only if the ANOVA test shows statistically significant differences between the experimental groups). Differences were assumed significant at the significance level of 0.05.

3. Results

FWW treatment involved two pathways (Experiment 1 and Experiment 2) differentiated by the presence or absence of the freezing step (temperature pretreatment).

3.1. Experiment I

We observed a continuous increase in SCOD after 96 h of the experiment, which ranged from 1238 to 1447 mg dm^3 O_2 for moving beds MBBR and SBR, respectively (Fig. 4A). The fixed bed reactors, FBR and RFBR, reached maximum SCOD of 1460 to 1370 mg dm^3 O_2, respectively, after 72 h of the treatment (Fig. 4 A). BOD_5 values showed a similar tendency (Fig. 4B). The increase in SCOD and BOD_5 may be explained by intense hydrolysis of suspended tissue fractions, in which the cells were not completely destroyed by homogenization. The hydrolysis outscored the treatment efficiency until 72 h of the experiment, and later the values of both parameters dropped significantly (Fig. 4 A, B).

The content of PO_4^{3-} and NH_4^+ increased in 72 h and 96 h of purification (Fig. 4 C, D). Then, the concentration of PO_4^{3-} dropped to 37–29.6 mg dm^3 after 96 h and NH_4^+ to 98.7–118 mg dm^3 after 72 h (Fig. 4 C, D). In both cases, the concentrations exceeded the values determined for raw FWW and were due to the hydrolysis.

In raw FWW average values of COD_{tot} ranged from 1480 to 1840 mg dm^3 O_2, and of SCOD from 461 to 573 mg dm^3 O_2 (Fig. 5). These differences indicated a significant amount of suspended solids – a fraction that undergoes hydrolysis causing a release of easily soluble organic contaminants and an increase in SCOD (Fig. 4 A, B).

Our study identified the RFBB reactor as the most efficient if FWW purification. Statistical analysis of the differences between bioreactors in the first pathway allowed for selecting this type of reactor as significantly excelling the other in SCOD and BOD_5 reduction (Tables 1 and 2).

3.2. Experiment II

Temperature pre-conditioning at - 40 °C significantly lowered the differences between COD_{tot} and SCOD in raw FWW, which then ranged from 1142 to 1164 mg dm^3 O_2 and from 963 to 954 mg dm^3 O_2 respectively (Fig. 6).

Thanks to this low-temperature pretreatmen we managed to achieve the maximum level of SCOD and BOD_5 in raw wastewater (Fig. 7 A, B). During 72 h the average values of SCOD and BOD_5 dropped over two times to 429-185 mg dm^3 O_2 and 294-145 mg dm^3 O_2, respectively (Fig. 7 A, B). The hydrolysis did not affect the wastewater treatment as negatively as in the first experiment. Concentration of the investigated

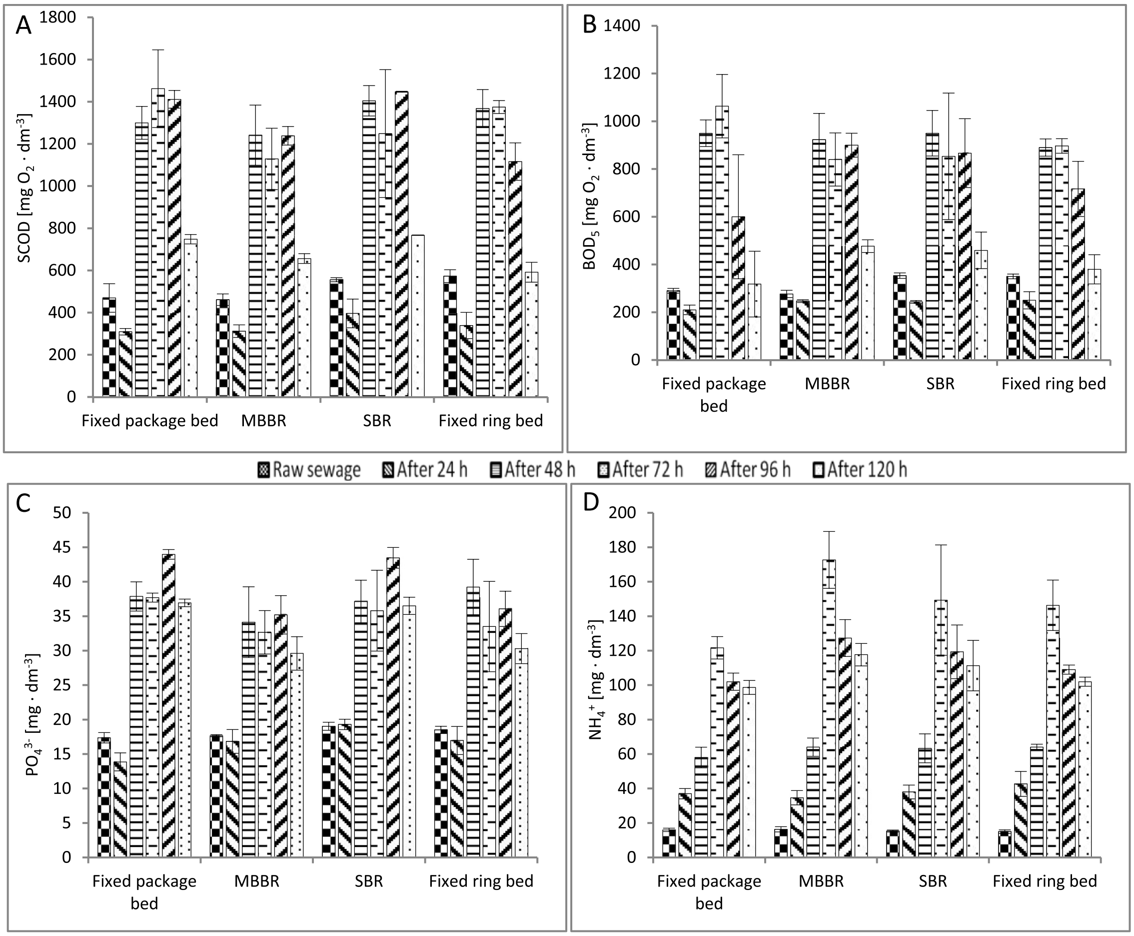

Fig. 4. A. SCOD, B. BOD_5, C. PO_4^{3-}, and D. NH_4^+ reduction during wastewater treatment in experimental bioreactors. AVG ± SD.

nutrients also showed a gradual decrease to 16.46–18.36 mg dm^3 for PO_4^{3-} and 60.37–79.4 mg dm^3 for NH_4^+ (Fig. 7 C, D). For phosphate ions, the decrease was 23–34%, and for ammonium ions it was 29–47%.

The analysis of date demonstrated that RFBR bioreactor was the most effective in FWW treatment (Tables 1 and 2), (Fig. 7 A, B).

Microscopic analysis confirmed proper quality of the biofilm

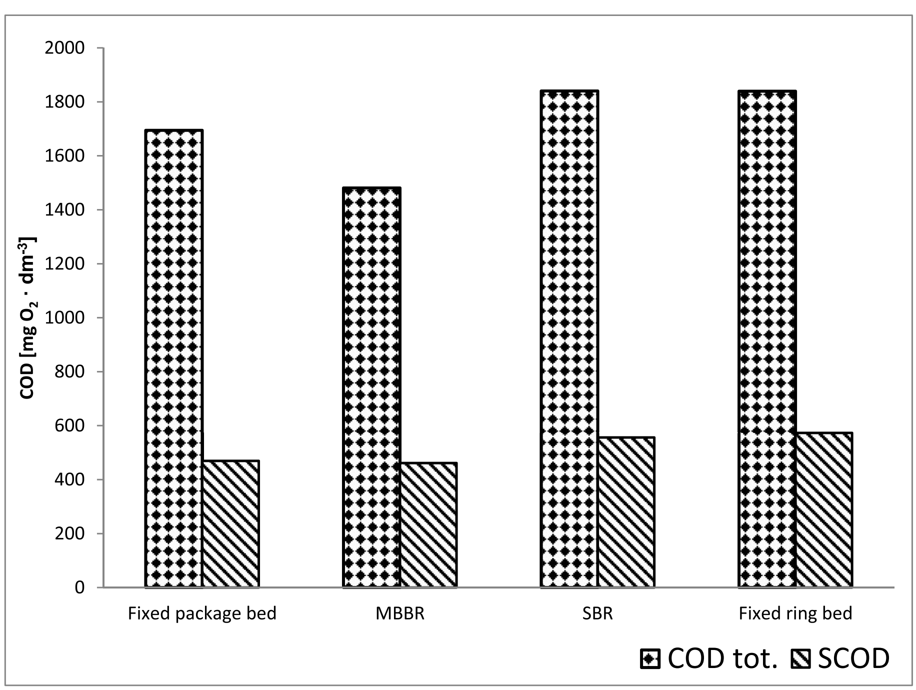

Fig. 5. Comparison of COD_{total} and SCOD in the same raw wastewater before treatment. AVG ± SD.

Table 1
ANOVA: One-dimensional significance tests for SCOD, BOD_5, PO_4^{3-}, and NH_4^+ (parameterization with sigma-limitations). Decomposition of effective hypotheses for two research campaign.

			Effect	SS	Degrees of freedom	MS	F	p
First research campaign	organic pollutions	SCOD	Free term	5725171	1	5725171	7040.859	0.000000
			Variables	60437	3	20146	24.775	0.0002
			Error	6505	8	813		
		BOD_5	Free term	2002876	1	2002876	273.8000	0.000000
			Variables	48865	3	16288	2.2267	0.162
			Error	58521	8	7315		
	nutrients	PO_4^{3-}	Free term	13333	1	13333	4262.1204	0.000000
			Variables	138.3	3	46.126	14.744	0.0012
			Error	25.026	8	3.128		
		NH_4^+	Free term	138460	1	138460	1978.001	0.000000
			Variables	678	3	226	3.233	0.081
			Error	560	8	70		
Second research campaign	organic pollutions	SCOD	Free term	1332000	1	1332000	771.6	0.000000
			Variables	104176	3	34725	20.1	0.0004
			Error	13809	8	1726		
		BOD_5	Free term	746005.3	1	746005.3	728.8767	0.000000
			Variables	43714.7	3	14571.6	14.2370	0.0014
			Error	8188	8	1023.5		
	nutrients	PO_4^{3-}	Free term	3758.418	1	3758.418	847.8910	0.000000
			Variables	11.154	3	3.718	0.838	0.509
			Error	35.461	8	4.433		
		NH_4^+	Free term	60760.68	1	60760.68	334.8268	0.000000
			Variables	820.01	3	273.34	1.5062	0.285
			Error	1451.75	8	181.47		

overgrowing the biological bed carriers. The results of random measurements showed the biofilm thickness to vary from 135 to 800 μm, with average of 430 μm (Fig. 8). Table 2 presents examples of morphometric measurements of the active surface of the biological bed (Table 3).

Microscopic analysis was crucial for assessing if the beds are properly covered with microorganism biomass before they were used in the experimental study of wastewater treatment.

4. Discussion

The study examined treatment effectiveness of wastewater from fish processing, and more specifically the washings from cutting stations. Average level of SCOD in this type of FWW is 800–2000 mg $O_2 \cdot dm^{-1}$ and the share of BOD_5 is exceptionally high (75–80% of SCOD). In the first experiment, the treatment processes seemed highly unstable. The analysis of SCOD and BOD_5 showed clearly that in the first days of the process, the organic loads increased gradually. The average differences of 321–361% between loads of SCOD and COD_{tot} in raw effluents indicated that a significant amount of organic matter remains on the filters during the preparation of the sample for laboratory measurements. Progressive increase in SCOD in the first days of the process was due to intense hydrolysis of dead cells in the homogenized substrate (Nogaj et al., 2015; Tay et al., 2004). This increase resulted from a direct release of easily soluble organic substances from the suspended matter into the FWW mixture. The purification was carried out at 24 °C, and this temperature accelerated the hydrolysis (Chowdhury et al., 2010). The increase in BOD_5 was also related to this phenomenon. Decomposition of insoluble organic fraction was also the reason for the increase in ammonium ion content in the wastewater up to 76 h and phosphate ions up to 96 h of the treatment (Calli et al., 2005; Aloui et al., 2009; Riaño et al., 2011; Queiroz et al., 2013). The experiment showed 194–253% growth in PO_4^{3-} and 637–780% in NH_4^+ concentration in the treated substrate, which significantly disturbed the C:N:P ratio of wastewater. Therefore, microorganisms in the biological beds were unable of incorporating the pollutants into their biomass (Lopes et al., 2000). During nitrification, concentrations of these ions constantly increased. Only 16% and 3–7% decrease in PO_4^{3-} and NH_4^+

Table 2
Tukey's HSD tests for selected studied parameters.

	Approximate probabilities for post hoc tests Error: MS intergroup = 813.14, df = 8					Approximate probabilities for post hoc tests Error: MS intergroup = 3.1283, df = 8				
First research campaign	SCOD		MBBR	SBR	RFBR	PO_4^{3-}		MBBR	SBR	RFBR
		FBR	0.018	0.843	0.0008		FBR	0.004	0.989	0.007
		MBBR		0.006	0.090		MBBR		0.006	0.960
		SBR			0.0004		SBR			0.011
		Approximate probabilities for post hoc tests Error: MS intergroup = 1722.2, df = 8					Approximate probabilities for post hoc tests Error: MS intergroup = 1023.5, df = 8			
Second research campaign	SCOD		MBBR	SBR	RFBR	BOD_5		MBBR	SBR	RFBR
		FBR	0.004	0.989	0.007		FBR	0.999	0.941	0.004
		MBBR		0.006	0.960		MBBR		0.933	0.004
		SBR			0.011		SBR			0.002

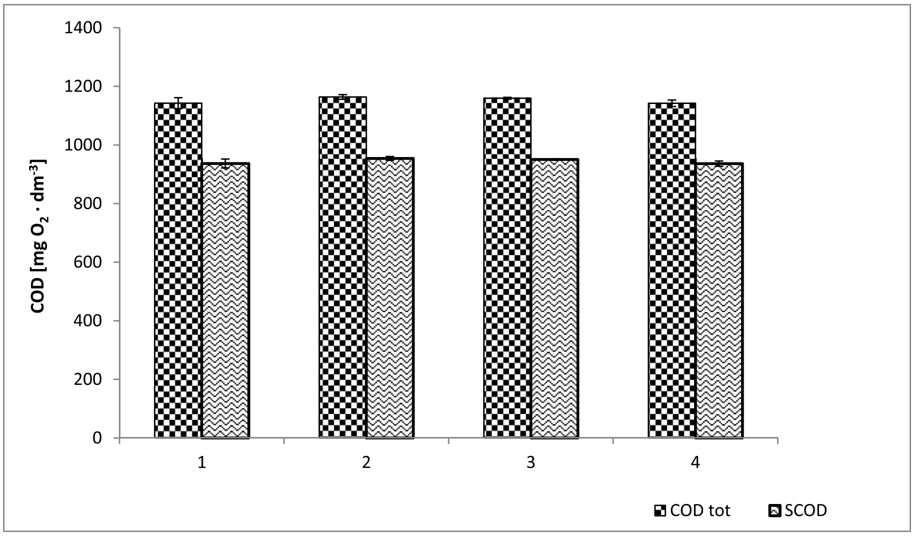

Fig. 6. The comparison of COD_{total} and SCOD in the same raw wastewater before treatment. AVG ± SD.

levels, respectively, was observed on the last day after 120 h of the experiment. The five-day period of purification may be critical for the selection of biological bed technology for FWW (Chowdhury et al., 2010).

In experiment 2, the raw homogenate was slowly frozen down to – 40 °C for a total destruction of cell membranes. This time, the filtered samples of wastewater revealed significantly higher organics loads. COD_{tot} values were only on average 22% higher than SCOD ones. Treatment efficiency improved significantly and a successive decrease in organic loads was observed from the beginning of the process up to 72 h.

We found out that the concentration of the tested nutrients in raw FWW in experiment 2 was the highest, with PO_4^{3-} ranging from 24 to 25 mg dm^{-3} and NH_4^+ from 110 to 115 mg dm^{-3}. A reduction of phosphates and ammonium reached 24–34% and 29–47%, respectively, for all types of bioreactors. The second experiment definitely confirmed

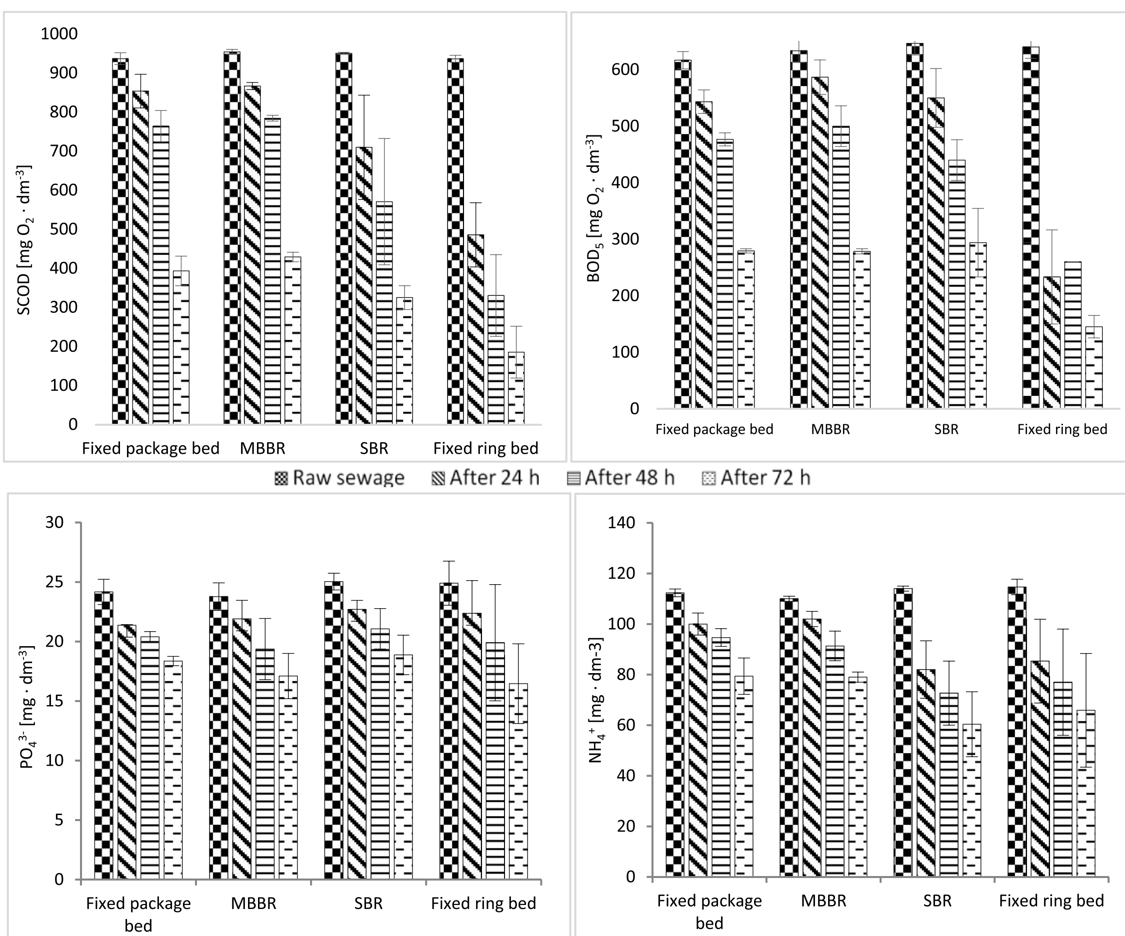

Fig. 7. A. SCOD, B. BOD_5, C. PO_4^{3-}, and D. NH_4^+ reduction during wastewater treatment in experimental bioreactors. AVG ± SD.

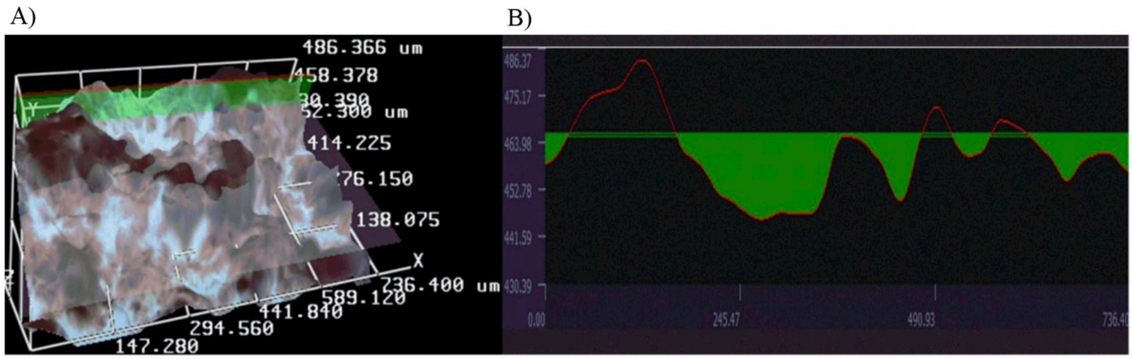

Fig. 8. Thickness distribution and character of the biofilm surface in an exemplary cross-section.

Table 3
Example of trend-line morphometric parameters evaluated samples of biofilm growing on (MBBR) carriers.

Value	Volume µm^3	Surface area µm^2	Specific surface area µm^2	$S_a \cdot SS_a^{-1}$
Min	126031.4	9254.4	8737.5	1.06
Max	640031.4	52166.7	53193.6	0.98
AVG	126031.4	30710.5	33133.1	0.93
SD	363452.9	15171.8	14184.9	0.04
Number of samples	10			

our assumption that the hydrolysis would affect the treatment kinetics observed in the first experiment (Lopes et al., 2000; Chowdhury et al., 2010). A comparison of the reactor effectiveness depending on the biological bed type did not provide a clear guideline as to which type can be used on a technical scale (Afonso and Bórquez, 2002). In both experiments, the fixed ring beds and SBR bioreactors showed the higher efficiency of wastewater treatment, as confirmed by statistical analysis. The times of wastewater treatment obtained for laboratory models are unacceptable on a technical scale, as with high amounts wastewater, the reactor chambers would have to be huge. The most critical condition here was the intense hydrolysis that significantly affected the physical model of the treatment. Due to the hydrolysis, the treatment process could not be designed in the flow system (Grady et al., 2011). Temperature conditioning of FWW is also unrealistic on a technical scale due to design challenges and high costs. The biological beds maintained a stable biomass level (biofilm on carriers surface). During the entire treatment process, the substrate did not affect the biomass quality in the biofilm (Table 2) on the carriers or in the flocks of the activated sludge. Due to high load of an easily biodegradable organic fraction, the microflora was dominated by microorganisms with low affinity to the substrate specific for biological beds in municipal wastewater treatment plants (Cloete and Muyima, 1997).

5. Conclusions

- FWW often requires preliminary conditioning to accelerate and increase the efficiency of subsequent treatment in biological bioreactors.
- Preliminary pretreatment should ensure maximum homogenization of raw wastewater to minimize the negative impact of hydrolysis.
- Microfiltration may be useful for complete elimination of organic suspended mater fraction in effluents.
- Only the easily soluble organic fractions should be directed to sufficiently aerated biological beds.
- Bioreactors with fixed biological beds seem promising in utilization of effluents from fish processing.
- Kinetics of purification should also be investigated in relation to different temperature ranges.

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